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**RESEARCH PAPER**

# Contribution of ammonium to stimulation of smooth pigweed (*Amaranthus hybridus* L.) germination by extracts of hairy vetch (*Vicia villosa* Roth) residue

JOHN R. TEASDALE* and PARTHAN PILLAI
*United States Department of Agriculture, Agricultural Research Service, Sustainable Agricultural Systems Laboratory, Beltsville, Maryland, USA*

Hairy vetch is a leguminous winter annual cover crop that provides a significant contribution toward meeting the nitrogen requirement of succeeding crops. Hairy vetch residue is capable of suppressing weeds, but low levels of residue can intermittently stimulate the emergence of weeds, particularly smooth pigweed. This research was conducted to assess the inhibitory and stimulatory effects of hairy vetch extracts on two smooth pigweed lots with differing dormancy conditions under differing germination conditions (25 or 35°C in light or dark). Full-strength extracts inhibited germination of both lots under all conditions, a result explained by the inhibitory osmotic potential of the full-strength extract. At ≤ 0.1× proportions of the hairy vetch extract, there was a slight stimulation of germination above that of the control (average = 11%) of both lots of pigweed under all germination conditions, except for a large stimulation (87%) by the more dormant lot at 25°C in light. A similar response to ammonium hydroxide solutions was observed, in which germination stimulation averaged 7% for all conditions except for germination of the dormant pigweed lot that was stimulated 115% by 15 p.p.m. of ammonium ($NH_4^+$) at 25°C in light. As the $NH_4^+$ concentration in the hairy vetch extract was similar to that in the ammonium hydroxide solutions that promoted the largest stimulation of germination, and because there was a high correlation between the degree of germination stimulation by hairy vetch extracts and by ammonium hydroxide, $NH_4^+$ appears to be the principle ingredient in the extract responsible for stimulation of smooth pigweed germination.

**Keywords:** allelopathy, cover crop, light, osmotic potential, temperature, weed.

## INTRODUCTION

Hairy vetch has become an important cover crop because of its winter hardiness, vigorous growth and high nitrogen (N) content. Early research in the southeastern United States of America (USA) focused on the N contribution of hairy vetch and other legume cover crops to grain crops (Smith *et al.* 1987). As hairy vetch has a N content of 3–4% and a low carbon : N ratio of 10–14, it provides a rapid input of mineralized N to soils (Smith *et al.* 1987; Kuo *et al.* 1997; Rosecrance *et al.* 2000). Under humid conditions, a significant portion of available N is released from hairy vetch residue within weeks after vetch is killed (Wagger 1989; Ruffo & Bollero 2003). Nitrogen contributions from a hairy vetch cover crop can substantially reduce the fertilizer N requirement for high N-requiring crops, such as corn (Smith *et al.* 1987; McVay *et al.* 1989; Decker *et al.* 1994). However, rapid mineralization of N can result in losses to the environment, both from leaching of inorganic N ions or losses of nitrous oxide after denitrification (Rosecrance *et al.* 2000).

A hairy vetch cover crop can make numerous other contributions to cropping systems including improved soil moisture infiltration and conservation (McVay *et al.* 1989; Clark *et al.* 1995), enhanced biological activity (Rothrock *et al.* 1995; Zablotowicz *et al.* 1998), control of selected pests and diseases (Rothrock *et al.* 1995; Mills

**Correspondence to: John R. Teasdale, USDA-ARS, Building 001, Room 245, Beltsville, MD 20705, USA.*
*Email: teasdale@ba.ars.usda.gov*

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*et al*. 2002) and weed suppression (Williams *et al*. 1998; Teasdale & Mohler 2000). Increasing amounts of residue of hairy vetch on the soil surface reduces emergence of annual weeds according to a negative exponential function (Teasdale & Mohler 2000). Weed suppression by cover crop residue has been explained by physical interference of mulch material with seedling emergence (Teasdale & Mohler 2000), by alteration of the soil microenvironment to enhance dormancy of weed seed in soils (Teasdale & Mohler 1993) and by the release of allelopathic chemicals that inhibit germination or growth processes (White *et al*. 1989).

Research with hairy vetch extracts and incorporated residue has demonstrated the potential for this cover crop to inhibit several weed and crop species (White *et al*. 1989). Relatively little research has been conducted to identify allelopathic substances in hairy vetch. Kamo *et al*. (2003) reported that allelopathic activity of nine-day-old hairy vetch seedlings was due to cyanamide. Bradow (1993) identified volatile ketones emitted by legume cover crops, including hairy vetch, that could inhibit cotton. Candole and Rothrock (1997) determined that ammonia ($NH_3$) released by hairy vetch into the soil atmosphere could account for suppression of the pathogen *Thielaviopsis basicola*.

In addition to inhibitory influences, hairy vetch residue also has stimulated weed emergence and growth (Mohler & Teasdale 1993; Gallagher *et al*. 2003). Weed stimulation by hairy vetch was intermittent in these studies, observed in one year but not in another. Pigweed (*Amaranthus*) species seem to be particularly prone to intermittently enhanced emergence in hairy vetch residue (Teasdale & Mohler 2000; personal communication from local extension personnel). Stimulation could be explained by several mechanisms including retention of soil moisture by surface residue cover, stimulation of growth by released N and N participation in breaking seed dormancy. Consequently, research was conducted to investigate the impact of hairy vetch extracts on two lots of smooth pigweed that differ in dormancy in order to identify conditions that might explain intermittent stimulation in the field. Associations between smooth pigweed response and inorganic N content and osmotic potential of extracts were explored as potential explanations.

## MATERIALS AND METHODS

Vegetative foliage of four cultivars of hairy vetch (Common, AU Early Cover, Auburn Population 8 and Auburn Population 26) was collected from field plots at Beltsville, Maryland, USA (Teasdale *et al*. 2004). Plant material was oven-dried at 52°C for two weeks, shredded, and extracts were prepared by shaking the plant material in water at the ratio of 1 : 10 (1 g dry material per 10 mL water) for 24 h on a platform shaker. Extracts were suctioned through filter paper (Whatman International, Maidstone, UK; No. 5) and stored at 4°C.

Seed from two smooth pigweed populations with differing germination requirements (designated lots 120 and 211) were collected locally at Beltsville and stored at –20°C. One hundred seed were placed in a Petri dish with filter paper (Whatman International, Maidstone, UK; No. 3) and 5 mL of treatment solution, and dishes were sealed with parafilm. Solutions consisted of a series of dilutions of the original extract yielding proportions of 1, $10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$, $10^{-6}$ and 0× the full-strength extract. As the response to the two lowest proportions was similar to that of the control, only data from the $10^{-4}$ and higher proportions are presented. Petri dishes were placed in a germination chamber with 24 h of incandescent plus fluorescent light (225 mol $m^{-2}$ $s^{-1}$) or dark illumination regimes, 25 or 35°C temperature regimes and 60% relative humidity. Petri dishes were placed in the chamber in a completely randomized design with four replications of each treatment. Experiments were conducted in a single chamber with the differing light and temperature regimes imposed in sequential experiments. Experiments with each set of environmental conditions were repeated once. After five days, the number of germinated seeds were determined and the shoot and root lengths of five randomly selected seedlings per dish were measured.

Solutions of ammonium hydroxide (29% $NH_3$, wt/wt) were prepared with total ammonia concentrations of 0, 1.5, 5.0, 15, 50 and 150 p.p.m. These concentrations represent total ammonia species without regard for the equilibrium between un-ionized $NH_3$ and $NH_4^+$ ion. One hundred seed were placed in a Petri dish with filter paper and 5 mL of ammonium hydroxide solution. Experimental procedures were conducted as described above for hairy vetch extract experiments. Ammonium and nitrate ($NO_3^-$)/nitrite in the hairy vetch extracts were determined by diluting the extract by one-tenth and analyzing on a Lachet flow-injection analyzer (Zellweger Analytics, Milwaukee, WI, USA) using standard techniques (Mulvaney 1996).

Aqueous solutions of polyethylene glycol-8000 were prepared at water potentials of 0, –30, –60, –100, –300, –600, –1000, –1500, and –2000 kPa (Michel 1983). One hundred seed were placed in a Petri dish with filter paper and 5 mL of polyethylene glycol solution. Exper-

imental procedures were conducted as described above for hairy vetch extract experiments. The water potential of hairy vetch extracts and polyethylene glycol solutions were measured using a dewpoint hygrometer (Wescor Logan, UT, USA; HR 33T) with sample chambers.

Data were subjected to analysis of variance using a mixed-model procedure (PROC MIXED, SAS version 8.2, SAS Institute, Cary, NC, USA). As a result of the high precision of these experiments and low variance of treatment means, almost every main effect and interaction were significant ($P < 0.05$). As not all of these significant effects were biologically meaningful, the authors have used their judgment to determine biological significance. In graphical presentation of data, standard error bars are not shown because most standard errors were equivalent to or smaller than the width of the symbols used to designate the means.

## RESULTS

The dormancy status of the two smooth pigweed seed lots used in these experiments varied as evidenced by the response to environmental conditions in the absence of extracts (Fig. 1). At 25°C, the germination of seed lot 120 was higher than that of lot 211 under both light and dark conditions. Both lots showed higher germination in light (average = 50%) than in dark (average = 34%) at 25°C. Both lots had higher germination rates at 35°C (average = 77%) than at 25°C (average = 42%) but there was little difference in germination between lots at 35°C. Other researchers also have found optimum germination for pigweed species at 35°C (Ghorbani *et al.* 1999; Steckel *et al.* 2004). Gallagher and Cardina (1998a) showed that the light requirement for germination was higher at 20°C than 30°C, confirming our result that there was a greater light requirement at the lower temperature.

There was little difference in germination or growth of smooth pigweed in response to extracts of the different hairy vetch cultivars (data not shown). The dose–response pattern to the range of extract proportions of all hairy vetch cultivars was similar. Consequently, data shown are averaged over all cultivars.

Full-strength hairy vetch extracts almost completely inhibited germination of both smooth pigweed lots under all conditions (Fig. 1). Hairy vetch extracts at proportions of ≤ 0.1 had little effect or caused a slight increase in smooth pigweed germination under most conditions. The most striking deviation from this pattern was an 87% increase in germination of lot 211 at 25°C in light at the 0.1 extract proportion (Fig. 1). This result demonstrates that a unique combination of conditions is required to achieve a substantial stimulation of smooth pigweed germination. This unique set of requirements could provide a basis for understanding the intermittent stimulation of emergence observed in the field.

Smooth pigweed germination of both lots under all conditions was inhibited by 150 p.p.m. of total ammonia species in ammonium hydroxide solutions (Fig. 2). This concentration of ammonium hydroxide had a pH of 8.5 and would be expected to dissociate into a majority of $NH_4^+$ ions and a small, but significant, amount of un-ionized $NH_3$ (Megie *et al.* 1967). Megie *et al.* (1967) showed that the un-ionized $NH_3$ in concentrated ammonium hydroxide solutions with basic pH was sufficient to inhibit seed germination and seedling growth of cotton. Therefore, inhibition of smooth pigweed germination by the high concentration of ammonium hydroxide in our experiments is probably also due to

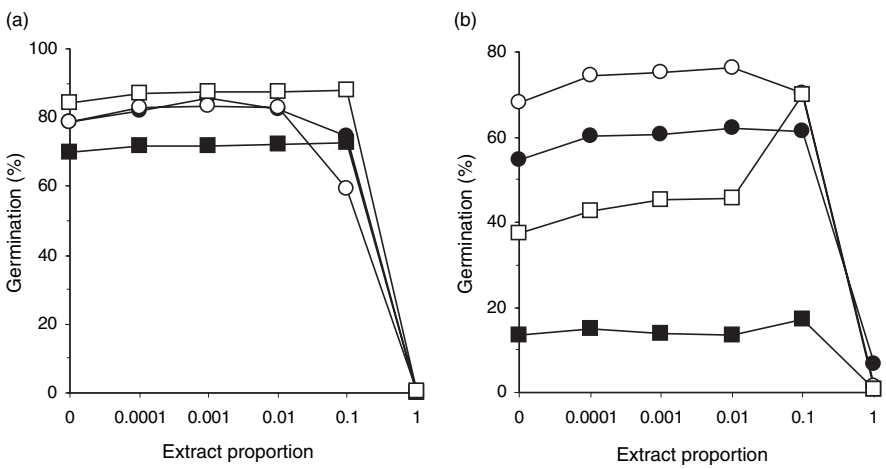


**Fig. 1.** Germination of two lots of smooth pigweed in response to proportions of full-strength hairy vetch extract at (a) 35°C or (b) 25°C and in continuous light or dark conditions. Standard error bars are not shown as they were similar to or less than the width of the symbol designating the mean. (●) lot 120, dark; (○) lot 120, light; (■) lot 211, dark; (□) lot 211, light.

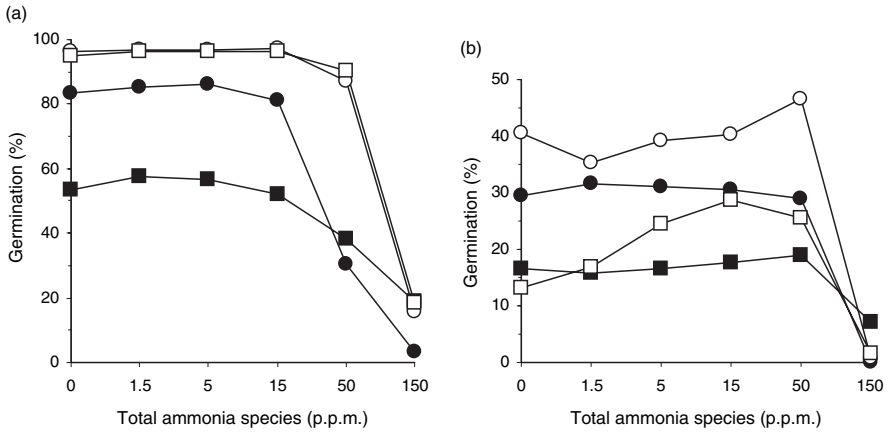


**Fig. 2.** Germination of two lots of smooth pigweed in response to the concentration of total ammonia species in ammonium hydroxide solutions at (a) 35°C or (b) 25°C and in continuous light or dark conditions. Standard error bars are not shown as they were similar to or less than the width of the symbol designating the mean. (●) lot 120, dark; (○) lot 120, light; (■) lot 211, dark; (□) lot 211, light.

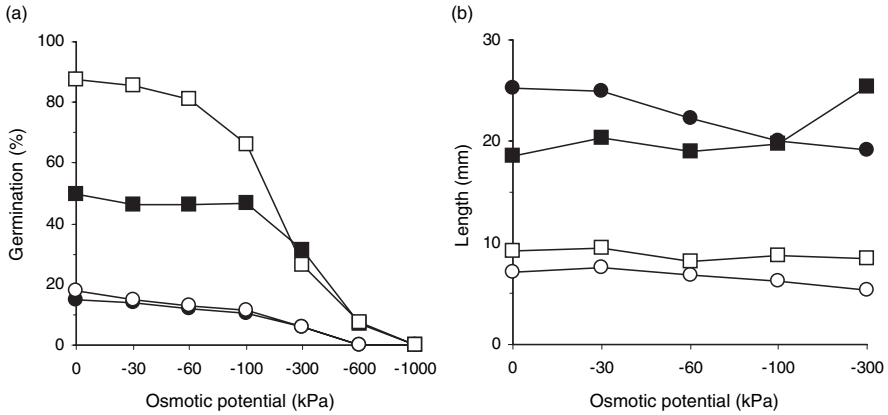


**Fig. 3.** (a) Germination and (b) root and shoot length of smooth pigweed in response to the osmotic potential established by polyethylene glycol solutions. Standard error bars are not shown as they were similar to or less than the width of the symbol designating the mean. (a): (●) 25°C, dark; (○) 25°C, light; (■) 35°C, dark; (□) 35°C, light; (b): (●) shoot, dark; (○) shoot, light; (■) root, dark; (□) root, light.

**Table 1.** Osmotic potential, ammonium and nitrate/nitrite concentration of hairy vetch extracts

| Extract proportion | Osmotic potential (kPa) | Ammonium (p.p.m.) | Nitrate/nitrite (p.p.m.) |
|---|---|---|---|
| 1.00 | −845 (35) | 169.0 (0.20) | 0.450 (0.150) |
| 0.10 | −170 (30) | 16.9 (0.07) | 0.045 (0.015) |
| 0.05 | −97 (9) | 13.9 (0.03) | – |

Standard deviations are shown in parentheses.

un-ionized $NH_3$. The $NH_4^+$ concentration in the full-strength hairy vetch extract was 169 p.p.m. (Table 1), suggesting that $NH_3$ toxicity could be involved in inhibition of smooth pigweed germination by the full-strength extract. However, the pH of this extract was 5.3. At neutral or lower pH, very little un-ionized $NH_3$ would be present, thus inhibition of smooth pigweed germination by hairy vetch extracts probably cannot be explained by $NH_3$ toxicity.

An osmotic potential of −600 kPa severely inhibited smooth pigweed germination under all conditions (Fig. 3a). This was a similar response to osmotic potential as that observed by Ghorbani *et al.* (1999). The osmotic potential of the full-strength hairy vetch extract was − 845 kPa (Table 1). Thus, the inhibition by the full-strength extract is probably explained by the high osmotic potential generated by the high concentration of solutes extracted. Osmotic potential also partially explained the phytotoxicity of hairy vetch extracts observed by White *et al.* (1989).

There was a small increase or no response in germination of smooth pigweed lots under most germination conditions at concentrations between 1.5 and 50 p.p.m. total ammonia (Fig. 2). However, a substantial stimulation of germination was observed with lot 211 at 25°C in light as total ammonia concentrations increased to 15 p.p.m.

(Fig. 2). Given that the concentration of $NH_4^+$ in the 0.1 dilution of hairy vetch extract was 16.9 p.p.m. (Table 1) and that the extract at this dilution was responsible for a similar large stimulation of lot 211 pigweed at 25°C in light (Fig. 1), this suggests that $NH_4^+$ could be the extract component responsible for this stimulation. Furthermore, the maximum stimulation of germination above the control for each lot under each set of germination conditions by hairy vetch extract and by ammonium hydroxide solutions followed a similar pattern (Table 2). These maximum stimulation values by hairy vetch extract and by ammonium hydroxide were highly correlated ($r = 0.978$), providing additional evidence that $NH_4^+$ could be the active extract component stimulating germination.

Smooth pigweed seedling root length was inhibited by hairy vetch extracts diluted to 0.1× proportion but pigweed shoot length was similar at this extract proportion to that of the controls (Fig. 4a). Growth responses of both lots of smooth pigweed were similar so data were averaged. There was little influence of ammonium hydroxide solutions or osmotic potential on pigweed seedling growth at concentrations that did not severely inhibit germination (Figs 3b, 4b). The $NH_4^+$ concentration and osmotic potential observed in the 0.1× proportion of hairy vetch extract (Table 1) were not sufficient to influence seedling growth according to the limited response to these factors demonstrated in figures 3b and 4b. Other compounds in the 0.1× proportion of extract must have been responsible for inhibiting smooth pigweed root growth. The absence of a large stimulation in pigweed seedling growth at lower proportions of hairy vetch extracts can be explained by the relatively high levels of $NH_4^+$ and low levels of $NO_3^-$ found in hairy vetch extracts (Table 1). Tyker *et al.* (1991) demonstrated that pigweed required the $NO_3^-$ rather than the $NH_4^+$ form of N for growth.

**Table 2.** Maximum stimulation of germination by hairy vetch extracts and ammonium hydroxide solutions

| Seed lot | Light | Temperature (°C) | Maximum stimulation of germination (%) | |
|---|---|---|---|---|
| | | | Extract | Ammonium hydroxide |
| 120 | dark | 25 | 13.1 (2.2) | 6.8 (7.1) |
| | | 35 | 8.9 (0.9) | 3.4 (1.7) |
| | light | 25 | 14.6 (4.0) | 14.5 (6.7) |
| | | 35 | 5.6 (1.1) | 1.1 (0.3) |
| 211 | dark | 25 | 29.1 (6.7) | 13.9 (7.2) |
| | | 35 | 4.4 (3.7) | 7.5 (4.1) |
| | light | 25 | 86.6 (7.2) | 115.0 (18.0) |
| | | 35 | 4.1 (2.4) | 1.3 (0.6) |

Maximum germination was determined at that concentration that gave the highest number of germinating seeds within each lot and set of light/temperature conditions. Percentage stimulation was computed by ((maximum germination − control germination)/(control germination)) × 100 for each lot and set of germination conditions. Standard deviations are shown in parentheses.

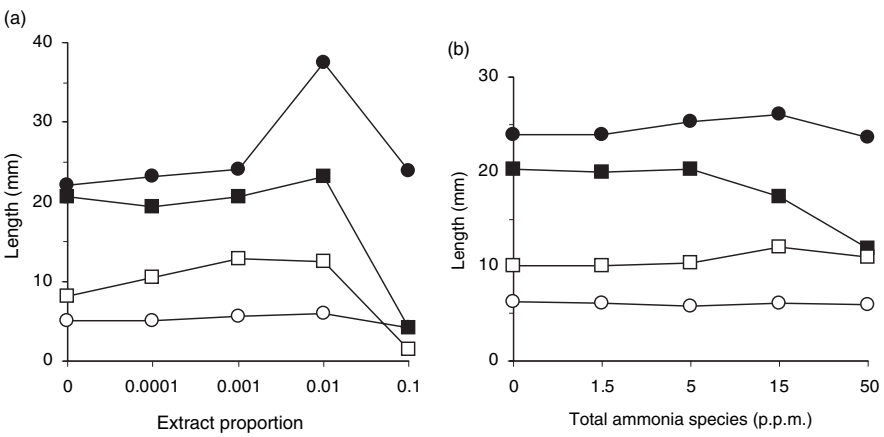


**Fig. 4.** Length of smooth pigweed shoots or roots in response to: (a) the proportion of full-strength hairy vetch extract and (b) the concentration of total ammonia species in ammonium hydroxide solutions. Standard error bars are not shown as they were similar to or less than the width of the symbol designating the mean. (●) shoot, dark; (○) shoot, light; (■) root, dark; (□) root, light.

## DISCUSSION

Our results showing that $NH_4^+$ is associated with the germination stimulation related to hairy vetch extracts complement the results of Gallagher and Cardina (1998b) regarding $NO_3^-$ stimulation of pigweed germination. These authors showed that $NO_3^-$ enhanced the very low fluence response that is responsible for pigweed seed germination in the presence of light. They also showed that $NO_3^-$ enhancement of light-dependent germination was greater at lower (20°C) than higher (30°C) temperatures. It is precisely under these conditions of low temperature (25°C) and light that lot 211 exhibited enhanced germination in the presence of hairy vetch extracts at stimulatory levels of $NH_4^+$. These results suggest that $NH_4^+$ might have a similar effect as $NO_3^-$ in promoting sensitivity of dormant seed lots to germination in light. Nitrate levels in these extracts were very low (Table 1), well below the amount used by Gallagher and Cardina (1998b), and probably had little influence on the results. Thus, inorganic N in the $NH_4^+$ form appears to be similarly effective as $NO_3^-$ at enhancing germination.

Several mechanisms have been presented by which inorganic N can break dormancy of weed seeds. Gallagher and Cardina (1998b) speculate that light-induced germination of pigweed species is associated with a transition from type II to type I phytochrome, as well as with phytochrome-independent gibberellic acid biosynthesis as soils warm in spring. They suggest that the presence of $NO_3^-$ sensitizes either or both of the phytochrome and gibberelic acid systems to enhance germination. Hendricks and Taylorson (1974) showed that $NH_4^+$ and $NO_3^-$ promoted germination of selected weed species. They suggested that $NH_4^+$ promoted germination of yellow rocket (*Barbarea vulgaris* R. Br.) and early wintercress (*Barbarea verna* (Mill.) Aschers.) by serving as a N source for metabolic processes limiting germination; whereas, $NO_3^-$ promoted germination of several species including tumble pigweed (*Amaranthus albus* L.) by inhibiting catalase which enhanced peroxidase activity. Cairns and deVilliers (1986) showed that $NH_3$ broke the dormancy of wild oat (*Avena fatua* L.) and several other grass species by increasing the permeability of the seed coat.

Hairy vetch residue has been shown to have a higher concentration of $NH_4^+$ than $NO_3^-$ in its biomass (Kuo *et al*. 1997), thereby providing an explanation for the higher levels of $NH_4^+$ than $NO_3^-$ in the extracts in our experiments (Table 1). However, soils that contain decomposing hairy vetch tend to have higher $NO_3^-$ than $NH_4^+$ levels (Hu *et al*. 1997; Sainju & Singh 2001). Despite this conversion to $NO_3^-$ in soils, $NH_4^+$ levels can be as high as 5 p.p.m. according to one report (Sainju & Singh 2001) and 15 p.p.m. according to another report (Hu *et al*. 1997). These levels of $NH_4^+$ would be sufficient to cause the promotion of germination of lot 211 smooth pigweed that was observed in this experiment at 25°C in light (Table 2).

The results of these experiments provide a hypothesis for the conditions required for the intermittent pigweed emergence that is frequently observed in the field. First, not all pigweed seeds respond, only specific cohorts of pigweed seeds with a dormant physiological configuration such as that in lot 211. Second, a response will occur when soil temperatures reach an intermediate temperature of ≈ 20–25°C, but not at higher temperatures. Third, hairy vetch residue levels on the soil surface need to be sufficiently low to transmit light and trigger a phytochrome response (this will occur with reasonable frequency at natural levels of hairy vetch as shown by Teasdale and Mohler (1993). Fourth, hairy vetch decomposition must yield $NH_4^+$ and/or $NO_3^-$ levels in the soil at required levels simultaneously with the above conditions. The intermittent observation of $NH_4^+$ at the required concentration in soils by Hu *et al*. (1997) and Sainju and Singh (2001) in some years but not others is consistent with the intermittent pattern of smooth pigweed emergence in a hairy vetch residue. Further research is needed to determine the relative roles of $NH_4^+$ and $NO_3^-$ in stimulating germination, and to monitor the correlation between patterns of these inorganic ions in soils and weed emergence in response to a hairy vetch cover crop.

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